

EFFECT OF SYMBIOTIC ZOOXANTHELLAE AND TEMPERATURE
ON BUDDING AND STROBILATION IN
CASSIOPEIA ANDROMEDA
(ESCHSCHOLZ)

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Many algal-invertebrate symbioses are based on mutual biotrophic benefit. The heterotrophic host is supplied with photosynthates from its phototrophic symbiont, and the latter obtains both nutrients and an optimal habitat (Smith *et al.*, 1969; Cook, 1972; Richmond and Smith, 1979).

Symbiotic associations were probably initiated by a chance meeting of the cosymbionts. A loose facultative symbiosis could evolve in time into a symbiosis obligatory for at least one of the partners. Such symbioses can affect both form and function of the cosymbionts, and through coevolution new species may have evolved (Margulis, 1976). The symbiogenic theory of the origin of mitochondria and chloroplasts is based on the above assumptions.

To illustrate such evolutionary trends, the following examples are usually cited: The platyhelminth *Amphiscolops langerhansi* is unable to achieve sexual maturity without its symbiotic algae (Taylor, 1971), and the closely related flatworm *Convoluta roscoffensis* is completely dependent on its symbiotic algae "for its bulk nutrients and growth stimuli" (Provasoli *et al.*, 1968). It has also been claimed that the scyphozoan medusa *Mastigias papua* will strobilate ephyrae only if infected with zooxanthellae, and "if a scyphistoma is deprived of its zooxanthellae, it can never strobilate" (Sugiura, 1964). Ludwig (1969) stated similarly that aposymbiotic scyphistomae of *Cassiopeia andromeda* obtained in his laboratory "have only a limited vitality, and cease their strobilation completely".

The above statements allege an obligate dependence of the hosts on their symbiotic algae. It is thus of interest that all the above invertebrates form aposymbiotic eggs and larvae, and the symbiotic algae are acquired anew at each generation.

In view of this, we reexamined the life cycle of *C. andromeda*. In this study we report strobilation and formation of aposymbiotic ephyrae by aposymbiotic scyphistomae, and the effect of temperature on budding and strobilation.

MATERIALS AND METHODS

Mature *Cassiopeia* medusae were collected from the Gulf of Eilat, brought to the laboratory within 8 hr, and maintained in aquaria at $18^{\circ} \pm 2^{\circ}\text{C}$. During transport and in the aquaria, the medusae released many aposymbiotic planulae. Planulae left in the aquaria, or maintained in water from the latter, developed into polyps hosting algae (Fig. 1a). In addition to the polyps obtained from medusae collected at Eilat, polyps were also obtained from a culture maintained in our department for several years.

* This study is part of a Master's thesis submitted by the junior author to the Hebrew University.

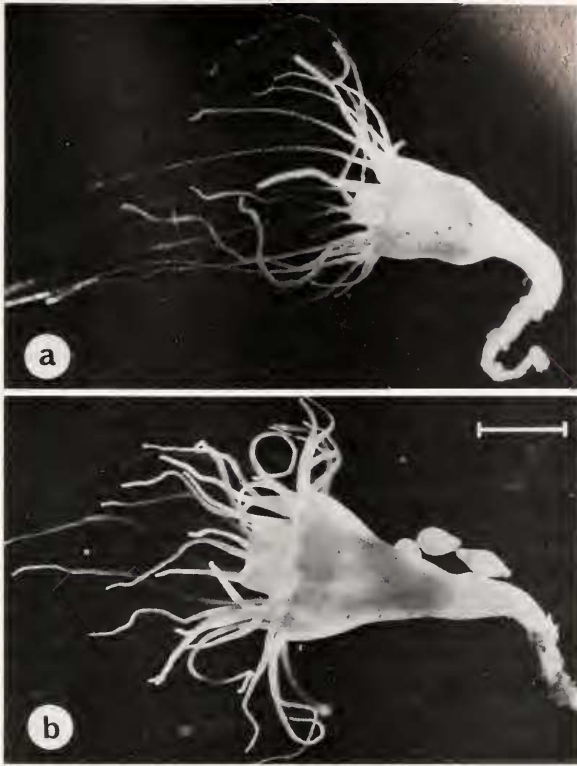


FIGURE 1. Polyps of *C. andromeda*. a. Symbiotic polyp. The dark dots in the calyx are endocellular zooxanthellae. b. Aposymbiotic polyp with three buds. Scale bar: 1 mm.

To obtain aposymbiotic polyps (Fig. 1b), planulae were collected from the aquaria, and washed and maintained in sterile sea water, in crystallizing dishes or finger bowls.

Aposymbiotic polyps were also obtained by incubation of symbiotic polyps in 10^{-6} M DCMU (3,3,4-dichlorophenyl, 1,1-dimethyl urea), for 35–40 days.

Polyps were fed $3\times$ a week to repletion, with 2–4-day-old *Artemia salina* larvae. The cultures were washed and media changed within 24 hr after feeding. Sea water (from the Mediterranean, salinity 37–39‰) was sterilized in an autoclave. Aposymbiosis of the various forms of *Cassiopeia* was verified by fluorescence microscopy. Stock cultures of polyps were maintained at $20^{\circ} \pm 2^{\circ}\text{C}$ under continuous illumination of 3–5 W/m^2 from white fluorescent lamps.

Crude homogenates of *Cassiopeia* were prepared from the oral lobes and sub-umbrellae of adult medusae, using a glass homogenizer. To reinfect aposymbiotic polyps, about 20 μl of fresh homogenate was injected into the polyp's mouth using a drawn-out Pasteur pipette. For "feeding" experiment controls, the same homogenates were heated for 30 min in a water bath at 45°C and stored at -18°C until used. Some homogenates were deep-frozen directly to -18°C , without prior heating.

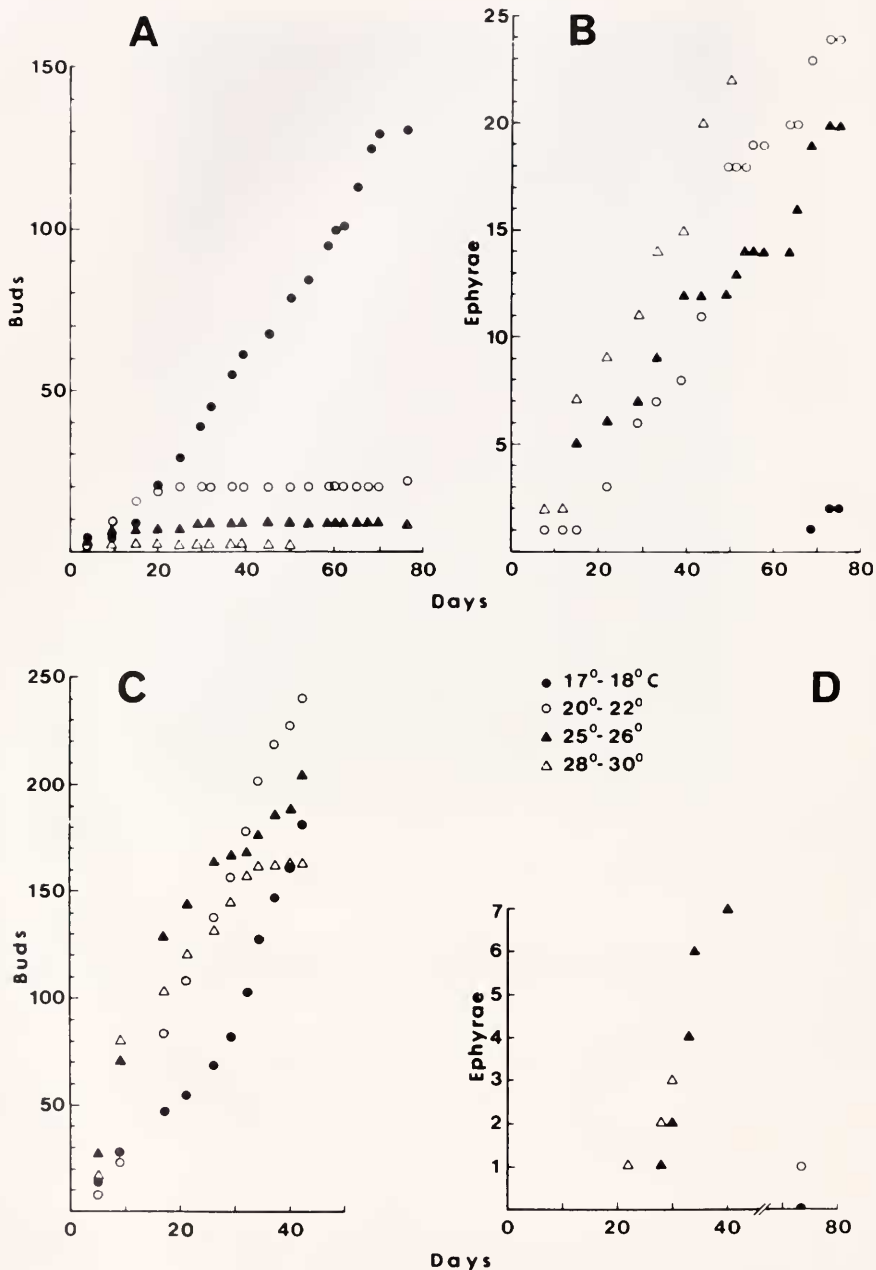


FIGURE 2. Effect of temperature on budding and strobilation of symbiotic and aposymbiotic *C. andromeda*. $N=7$. A and B, symbiotic polyyps. C and D, aposymbiotic polyyps.

RESULTS

Budding and strobilation of symbiotic polyyps

Groups of seven symbiotic polyyps were placed in crystallizing dishes at four different temperature ranges between 17° and 30°C. The numbers of buds and

TABLE I

Budding and strobilation of C. andromeda under various conditions.

| Polyps | °C | Buds/polyp | Ephyrae/polyp |
|--------------------------|---------|------------|---------------|
| Symbiotic* (N = 7) | 17°-18° | 19.6 | 0.3 |
| | 20°-22° | 3.1 | 3.4 |
| | 25°-26° | 1.0 | 2.8 |
| | 28°-30° | 0.3 | 3.1 |
| Aposymbiotic† (N = 7) | 17°-18° | 25.7 | 0 |
| | 20°-22° | 34.3 | 0.1 |
| | 25°-26° | 29.1 | 1.0 |
| | 28°-30° | 23.1 | 0.4 |
| Reinfected (N = 10) | 20°-22° | 2.9 | 2.0 |
| Injected (N = 10) | 20°-22° | 62.5 | 1.0 |

* Some symbiotic polyps formed up to five ephyrae in succession.

† Aposymbiotic polyps never formed more than one ephyrae. For time-rate of budding and strobilation, see Figures 2 and 4.

ephyrae formed at each temperature were recorded, as shown in Figure 2 (A and B) and Table I. At higher temperatures budding was fully arrested after 20–25 days, and only ephyrae were formed. Strobilation was about equal at all temperatures between 20° and 30°C. Sometimes, budding and strobilation occurred simultaneously, as was noted by Hoffmann *et al.*, (1978).

Budding and strobilation of aposymbiotic polyps

Experimental conditions for aposymbiotic polyps were identical to those described above for symbiotic polyps. Budding was equally frequent at all temperatures tested, and aposymbiotic ephyrae were formed at the higher temperatures (Fig. 2B, C; Table I). In this and similar experiments, a total of 26 aposymbiotic ephyrae (Fig. 3) were obtained from 130 aposymbiotic polyps at temperatures above 25°C. Only two ephyrae were obtained from several hundred aposymbiotic polyps below this temperature.

During strobilation, a yellow-green color appeared in the upper part of the calyx of the aposymbiotic scyphistomae. This color was also observed in the free-swimming young ephyrae, but it disappeared 7–10 days after their release.

Effect of reinfection and homogenate injection

Ten aposymbiotic polyps previously maintained at 18°C were reinfected with zooxanthellae by injection of a fresh homogenate into their coelenterons. The polyps were then maintained at 20°–22°C (Fig. 4A). Within 5 days, endosymbiotic algae could be seen with the naked eye. Strobilation could be seen on about the 10th day, and on the 18th day two ephyrae were released. All buds and ephyrae formed by the reinfected polyps contained zooxanthellae.

Ten similar aposymbiotic polyps maintained at 20°–22°C were injected every 2 days with a homogenate containing heat-killed algae (Fig. 4B). Strobilation was first observed on the 34th day, and the first ephyrae released on the 43rd day. The above experiments are summarized in Table I. In these and in similar "feeding" experiments, a total of 17 ephyrae and 2025 buds were obtained from

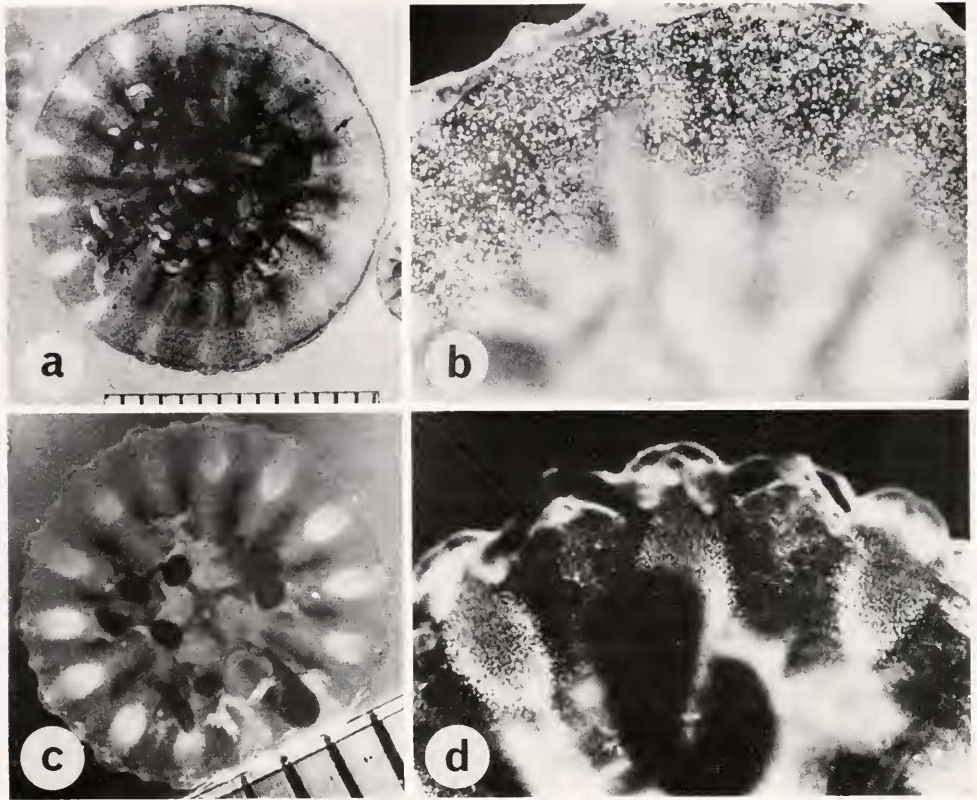


FIGURE 3. Symbiotic and aposymbiotic ephyrae of *C. andromeda*. a. Algae-hosting ephyrae: the dark central area is heavily infected with zooxanthellae. b. Enlarged margin of same: zooxanthellae are seen as bright dots through the transparent umbrella, on dark background. c. Aposymbiotic ephyrae: note transparent central area. The dark oral lobes contain a violet-blue or brown pigment. d. Enlarged margin of same: note transparent umbrella on dark background, and white clusters of nematocytes. In a and c, milimetric scale.

44 injected aposymbiotic polyps. Strobilations were also obtained in experiments in which homogenates with freeze-killed algae were used. There was no reinfection in any of the polyps.

DISCUSSION

This study shows that elevated temperatures and endosymbiotic algae act synergistically to induce strobilation in *C. andromeda*.

The direct effect of temperature on morphogenesis has been observed in both symbiotic and aposymbiotic polyps. In the former, there was a marked preference for bud formation at temperatures of 17°–18°C. At higher temperatures budding ceased completely and only ephyrae were formed. In the aposymbiotic polyps, budding rate was similar at all tested temperatures between 17° and 30°C. Strobilation, however, was definitely temperature dependent, and occurred almost only at above 25°C (Fig. 2, Table I).

Such a requirement for elevated temperatures to induce strobilation has also been reported for the symbiotic scyphozoan medusa *Mastigias papua* (Sugiura,

1965), and for the nonsymbiotic *Chrysaora quinquecirrha* (Loeb, 1972). In both cases, precooling to 20°C for several weeks was required for later strobilation at 22° and 26°C, respectively. The stock cultures of polyps used in our experiments were maintained at 20° ± 2°C, but we did not thoroughly investigate whether such precooling is required also for *C. andromeda*.

The morphogenic effect of endosymbiotic algae was shown by the lower temperatures at which strobilation occurred in the symbiotic polyps, as well as by the relative number of ephyrae and buds formed at each temperature (Table 1). The lowest temperature at which aposymbiotic strobilation occurred was 20°–22°C. Symbiotic polyps formed some ephyrae even at 17°–18°C.

We therefore looked for a strobilation-enhancing factor(s) in the endosymbiotic algae.

Reinfection of aposymbiotic polyps enhanced strobilation at 20°–22°C. Twenty ephyrae were formed in 60 days by 10 reinfected polyps (Fig. 4A), vs. only one ephyrae formed at the same temperature from seven aposymbiotic polyps after 73 days (Fig. 2D). A similar though less marked effect on strobilation was obtained by injecting preheated or frozen homogenates into aposymbiotic polyps. In one experiment (Fig. 4B), 10 ephyrae were formed by 10 aposymbiotic polyps in 60 days. At the same time, however, 600 buds were formed. This relatively high rate of budding correlates with the high budding rate shown in Figure 2C for aposymbiotic polyps. In all these experiments (Figs. 2, 4), initiation of strobilation was accompanied by a synchronous decrease in budding rate.

The lag time to the release of the first ephyrae was much shorter in the symbiotic polyps than in the aposymbionts (Fig. 2). A similar relation was found between the reinfected polyps and the aposymbionts injected with homogenates (Fig. 4).

From the results obtained in our study, we suggest that morphogenic processes in *C. andromeda* shift from budding to strobilation at relatively higher metabolic rates.

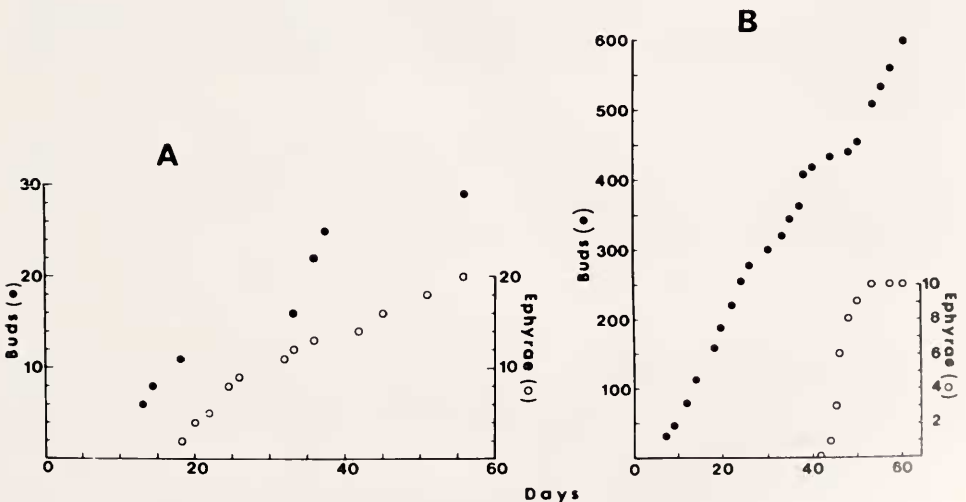


FIGURE 4. Formation of buds and ephyrae by aposymbiotic polyps. $N = 10$. $T^{\circ} = 20 \pm 2^{\circ}\text{C}$. A. Polyps reinfected with zooxanthellae on day 0. B. Polyps injected with heat treated homogenate of symbiotic mature medusae.

A higher metabolic rate can be obtained either by a rise in ambient temperature, or by a factor(s) that enhances metabolism. Such a factor is formed by *Cassiopsea* polyps, and it is probable that its accumulation enables strobilation of aposymbionts even at low temperatures. The formation of this factor by *Cassiopsea* is facilitated by a metabolite formed by the zooxanthellae. This algal metabolite is non-specific, as it is present also in non-strobilating symbiotic adult medusae.

Further experiments are required to elucidate quantitatively and qualitatively the characteristics and effects of the proposed factor(s) and its relation to effects of temperature and the metabolites of the algal symbionts.

The ecological implications of the above described combined effects of temperature and endosymbiotic algae can only be speculated upon. In its natural habitat in the Gulf of Eilat, *C. andromeda* can be found along the open coast, as well as in closed lagoons and mangroves. In the open coast the yearly temperature fluctuation is 17°–27°C, but in lagoons and mangroves a range of 13°–33°C has been recorded (Por *et al.*, 1977). Budding would thus be enhanced in winter, while at the higher summer temperatures ephyrae would be formed. In the sea, no aposymbiotic *C. andromeda* have yet been reported. By the ease at which algal infections occur in these polyps, it is doubtful whether aposymbiotic specimens ever occur in their natural habitat.

We do not know yet if aposymbiotic ephyrae can grow into sexually mature medusae, and thus complete an aposymbiotic life cycle in this species. In preliminary experiments we obtained a twofold increase in diameter of aposymbiotic ephyrae (from 5.1 to 11.5 mm), accompanied by normal morphological development.

Our observations on aposymbiotic strobilation in *C. andromeda* raise some questions with regard to the evolution of obligatory symbioses. Loss of capacity in a host to complete its life cycle in absence of its symbionts would indicate an evolutionary genetic integration in the sense described by Margulis (1976). It would thus be possible to claim that we have at our hands a new species, made up respectively of animal and algal tissues. Such an integration, however, should express itself also in the capability of the host to directly inherit its endosymbionts through the germ cells. In *C. andromeda*, as well as in *Mastigias papua*, *Amphiscolops langerhansii*, and *Convoluta roscoffensis*, this is not the case. Aposymbiotic larvae are formed by these species, and the symbiotic algae are acquired anew at each generation.

On the basis of our results that strobilation in *C. andromeda* is not dependent upon a symbiont, *vs.* the hypothesized obligatory dependence of the above species on their respective endosymbiotic algae, it would be of interest to reexamine the algae for the extent of host-symbiont integration. Such a study would add to our understanding of the effect of symbiosis on evolution.

SUMMARY

The role of the zooxanthellae in the life cycle of *Cassiopsea andromeda* was reexamined. Symbiotic and aposymbiotic polyps were maintained at various temperatures between 17° and 30°C, and numbers of buds and ephyrae formed at each temperature were recorded.

The number of buds formed by the symbiotic polyps was inversely related to temperature. After 20 days at above 20°C, only ephyrae were formed.

In aposymbiotic polyps, buds were formed at all temperatures tested. Strobilation occurred above 25°C, and aposymbiotic ephyrae were obtained. Aposymbiotic

polyps reinfected with zooxanthellae formed ephyrae at 22°C. A similar effect was obtained by repeatedly injecting aposymbiotic polyps with heat- or cold-inactivated homogenate derived from adult medusae. No reinfection occurred in the latter.

It is concluded: 1. At higher metabolic rates, strobilation supersedes budding in *C. andromeda*. 2. A higher metabolic rate is obtained at elevated temperatures, and by a biotrophic effect of the symbiotic zooxanthellae. 3. Symbiotic zooxanthellae are not essential for strobilation in *C. andromeda*.

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